Resistance and Sensitivity of Some Bacterial Strains Isolated from Hospital Wastewater and Nile Water Using Chlorination and Some Antibiotics in Cairo (Egypt)

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Abstract: A total of 394 bacterial isolates were isolated from Kasr AlAiny hospital wastewater effluent and river Nile water in Cairo (Egypt). Each 2 isolates from both sources were mixed in co-cultures and incubated (at room temperature) in sterilized Nile water for 48 hours under normal laboratory condition, taking into consideration the same gram staining and the different species. In addition, some isolates (before and after transfer of antibiotic resistance features tested) were exposed to chlorine dose (5ppm) for 120min at room temperature. The results showed that *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus faecalis* converted from antibiotics sensitive to antibiotics resistant when mixed with *Streptococcus faecalis*, *E. coli* and *Staphylococcus aureus*, respectively for Naldixic Acid (30 µg), Gentamycin (10 µg), Ampicillin (30 µg) and Amoxicillin (30 µg). In addition, these bacteria became more survival when treated with chlorine. On the other hand, the results demonstrated that these bacteria was sensitive when exposed to Erythromycin (15 µg), Tetracycline (30 µg), Lincomycine (15 µg) and Rifamipicin (50 µg). Moreover, S*almonella typhi* and *E. coli* isolated from Nile water were sensitive to chlorine (5 ppm) and antibiotics tested in both single and mixed cultures. Generally, the strains isolated from wastewater were more survival in chlorine dose tested than the bacterial isolates from Nile water samples. So, hospital effluent must be treated before delivering to Egyptian aquatic environment to protect public health.

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1. Introduction

Hospitals and cities sewage, as well as rivers and reservoirs polluted with secondary treated sewage contained large commonly used antimicrobial drugs. The survival of R^+ plasmid Coliform and the transfer of resistance during treatment of sewage and in polluted waters indicate that advanced treatment wastewater will be necessary to protect water resource against these organisms which have potential health signification **(Grabow, 1977 and Rijal *et al* 2009).** In addition, **Al-Ghazali *et al,* 1988** and **Munir *et al* (2011)** reported that sewage and wastes are the main sources of antibiotics resistant bacteria in the aquatic environment. Also, the escalating problem of emergence of antibiotic resistant bacteria and their resistant genes is becoming a major global health issue **(Chee-Sanford *et al,* 2001; Levy, 2002).**

Wastewater treatment plants are the main recipients of enteric microorganisms carrying antibiotic resistance genes. Microbial ecologists have emphasized the importance of waste water as a site for horizontal gene transfer, since it is a rich medium with high concentrations of various microorganisms (**Altherr & Kasweck, 1982**; **Mach & Grimes, 1982** and McPherson **& Gealt, 1986**). **(Reinthaler *et al,* 2003 and Silva *et al,* 2006).**

Andersen, (1993) found that in Copenhagen (*Denmark*), the bacterial resistance are transient characteristics, and resistance percentages may either decrease or increase following passage through the wastewater treatment plant (Antibiotic resistant genes and antibiotic resistant bacteria have been detected in wastewater samples **(Reinthaler *et al,* 2003; Pruden *et al,* 2006; Auerbach *et al,* 2007; Brooks *et al*, 2007 and Zhang *et al*, 2009a, b)**. Also, the release of antibiotic resistant organisms through wastewater effluents into streams had been previously reported **(Iwane *et al,* 2001 and Gallert *et al,* 2005). Iwane** and their colleagues reported approximately 8% and 6.7% of tetracycline resistant bacteria to be found in the pre- and post-chlorinated samples of a wastewater treatment plant respectively and then close to discharge location in the river water **(Iwane *et al,* 2001).** Also, the role of wastewater treatment plants in reducing the load of antibiotic resistant bacteria present in raw sewage is not well known **(Rijal *et al*, 2009).**

Due to miss use and up use of numerous antimicrobial agents in treatments of animal, human, and plant in worldwide practice providing both desirable and undesirable consequences such as exist of antibiotic resistant bacteria pathogens **(Aminov *et al*, 2001; Levy, 2002; Se´veno *et al*, 2002 and Peak *et al*, 2007).**

Some studies on the effect of chlorination in water treatments have shown to be conflicting; furthermore, have inspected the re-growth or reactivation of antibiotic-resistant bacteria after chlorination in wastewater **(Iwane *et al*, 2001; Gallert *et al*, 2005 and Jeppsson *et al*, 2007**). The presence of multiple-antibiotic-resistant bacteria from effluents of Wastewater Treatment Plants into natural waters could pose a serious problem as a secondary pollutant of drinking water (**Huang *et al*, 2011**). However, it has been suggested that certain conditions within the wastewater treatment plants might increase the number of antibiotic resistant bacteria during the treatment process **(Reinthaler *et al*, 2003 and Silva *et al*, 2006).**

The impact of disinfectants on antibiotic-resistant organisms has not been widely researched. Chlorination and ultraviolet (UV) disinfection are two of the most common disinfection processes currently used in water and wastewater treatment globally. Both of them have been proven to be effective against a range of waterborne pathogens, including *E.coli*, which is commonly used as an indicator organism **(Baumann and Ludwig 1962; Wilson *et al*, 1992 and USEPA, 1999).**

In addition, **Osman (1999)** found that it could be Trans the R+ plasmid between the different bacterial in aquatic environment especially the same shape and gram staining.

Also, given that some previous studies have shown the potential for water and wastewater treatment to select for the survival of antibiotic-resistant species over antibiotic-sensitive species, it is important to investigate whether antibiotic-resistant bacteria may be more resistant to disinfection processes **(Patrick & Rahn, 1976; Durbeej & Eriksson, 2003 and Hartl & Jones 2005)**.

The main aim of the present study was to transfer the features of antibiotics resistant bacteria which survive for 120 minutes in 5 ppm concentrated chlorine dose from Kasr AlAiny hospital effluent samples containing isolated bacteria to another set of bacterial strains isolated from Nile water in Cairo.

2. Materials and Methods

Sample collection

Every month, two types of water samples were collected during 2010. The first, Nile water samples were collected at inlet of El-Giza Water Treatment Plant and the second, raw wastewater (untreated sewage) were collected from Kasr AlAiny hospital effluent. The samples were collected in 2 liters sterile glass bottles and transferred to the lab in ice box.

Isolation and Identification

E. coli, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Streptococcus faecalis* were isolated using MPN (most probable number) as well as membrane filter technique methods according to **APHA (2005)**. Poured plates were incubated at 37 ºC for 24 hours, after incubation, randomly selected colonies with appropriate morphological characteristics were sub-cultured for enrichment and purification using specific agar media (**APHA, 2005**) for every isolate. The total count of isolates were 56, 48 for *E. coli*, 24, 18 for S*almonella typhi*, 59, 45 for *Pseudomonas aeruginosa*, 36, 42 for *Staphylococcus aureus*, and 32, 34 for *Streptococcus faecalis* from raw wastewater and Nile water samples, respectively.

Chromogenic media used for confirmed microbial isolation

Five different types of chromogenic agar media by Himedia (India) were used for confirming the strains which isolated by traditional methods (**APHA, 2005).** HiCrome UTI Agar (M1571, LOT WF256) for *E. coli*; HiCrome Improved Salmonella agar for S*almonella typhi*; *Pseudomonas* Selective Agar (M120) for *Pseudomonas aeruginosa* ; HiCrome *Aureus* Agar Base (M1468,) for *Staphylococcus aureus* and HiCrome Enterococci agar (M 1414) for *Streptococcus faecalis*.

Antibiotic assay

The sensitivity of the strains against various antibiotics were determined by using antibiotic sensitivity solution; Naldixic Acid (30 µg), Gentamycin (10 µg), Ampicillin (30 µg), Amoxicillin (30 µg); Erythromycin (15 µg), Tetracycline (30 µg), Lincomycine (15 µg) and Rifamipicin (50 µg). Mueller Hinton Agar (MHA) by Himedia (M173) was used to evaluate the bacteria tested for antibiotic resistance. One colony from pure culture (24 hours age grown on triptcase soy agar) putting onto 2 ml sterile distilled water tubes and vortex for 5 minutes. By sterile pipette 0.2 ml from these tubes putting onto Mueller Hinton Agar which contained antibiotic with the same concentration previously mentioned and incubated for 24 hours. After incubation, the present growth was resistant and the absent was sensitive as well as the plates control which contained Mueller Hinton Agar without antibiotic tested **(Andersen, 1993)**.

Transfer of resistance

The potential donor (isolates from hospital wastewater effluent samples) and the recipient (isolates from Nile water samples) were grown separately overnight in sterile nutrient broth in test tubes at 37 °C (APHA**, 2005)**. One ml (10^4 cfu) portion of both the donor and recipient culture was transferred to 100 ml sterile Nile water flask (200 ml capacity). Mating was allowed to proceed uninterrupted for 48 hours at room temperature. Separately, two strains were mixed with each other in a flask according to prescription of antibiotics resistant to sensitive (*E. coli* with S*almonella typhi*, *E. coli* with *Pseudomonas aeruginosa*, S*almonella typhi* with *E. coli*, S*almonella typhi* with *Pseudomonas aeruginosa, Pseudomonas aeruginosa* with *E. coli*, *Pseudomonas aeruginosa* with S*almonella typhi*, *Staphylococcus aureus* with *Streptococcus faecalis*, and *Streptococcus faecalis* with *Staphylococcus aureus*).

Chlorine dose

Chlorine water was obtained from the lab of El-Giza Water Treatment plant which used in this test. Three flasks (capacity 4 liters) each contained 3 liters of raw Nile water. Then the flasks were autoclaved, after autoclaving, one of them inoculated with 5 strains (*E. coli*, S*almonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*) which were isolated from Nile water samples. The second flask inoculated with 5 strains (transfer of resistance feature) which were isolated from Nile water samples (*E. coli*, S*almonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*). The third flask inoculated with five strains (*E. coli*, S*almonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*) isolated from wastewater effluent of Kasr AlAiny hospital. Taking into consideration the total counts were about 10^3 to 10^4 cfu / 100 ml for each of them with dose 5 ppm of chlorine dose, during 2 hours in the presence of aluminum sulfate (25-40 ppm) as flocculation and neutralization according to **APHA, (2005).** The samples were taken after 0, 10, 20, 30, 40, 60 and 120 minutes.

Statistical analysis:

The statistical analyses (the mean) were carried out according to procedure of **Snedcor and Chochran (1973).** The mean data values were using (MSTAT) computer software package version 2.1.

3. Results and Discussion

The results in **Table (1)** showed that, about 394 bacterial isolates were isolated from Kasr AlAiny hospital effluent and river Nile water. Two hundred and seven isolates were found in hospital effluent and one hundred eighty-seven were found in Nile water with percentage of 52.54 and 47.46% respectively. Gram negative Bacteria i.e. (*E. coli, Salmonella typhi and Pseudomonas aeruginosa*) were about 63.45% (250 isolates) and gram positive bacteria (*Staphylococcus aureus and Streptococcus faecalis*) were about 36.55% from the total numbers. The isolation was performed using specific chromogenic culture media as mentioned previous.

Table (1): The numbers of bacterial isolates from Kasr AlAiny hospital effluent and Nile water samples.

Isolates	Sources	
	Hospital effluent	Nile water
E. coli	**56**	**48**
Salmonella typhi	**24**	**18**
Pseudomonas aeruginosa	**59**	**45**
Staphylococcus aureus	**36**	**42**
Streptococcus faecalis	**32**	**34**

The results in **Table (2)** indicate that, the resistance to antibiotics for *E. coli* (56 isolates) isolated from Kasr AlAiny hospital effluent were low, varying from 1.9% for the antibiotic Lincomycine (one isolate) to 17.9% for the antibiotic Rifamipicin (10 isolates). In contrast, the sensitivity to all antibiotics tested were high varying from 82.1% (46 isolates) to 98.1% (55 isolates) for the same two antibiotics. On the same pattern, the resistance and sensitivity to antibiotics for *Salmonella typhi* (24 isolates) and *Pseudomonas aeruginosa* (59 isolates) isolated from the same source were similar to *E.coli*. The resistance to antibiotics for *Salmonella typhi* varying from 4.2% for Amoxicillin and Naldixic acid (one isolate) to 33.3% for Rifamipicin (8 isolates). The variation in resistance for *Ps. aeruginosa* ranged between 5.1 to 22% (3 to 13 isolates) for Tetracycline and Rifamipicin, respectively. While the sensitivity of *Salmonella typhi* and *Pseudomonas aeruginosa* for the same antibiotics were 66.7 – 95.8% and 78-94.4% respectively. The sensitivity of *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* isolated from the river Nile (111 isolates) for all antibiotics tested were high (91.6-100%), (94.4-100%) and (93.3-100%), respectively.

Table (2): Resistance and sensitivity of some bacteria isolated from both Kasr Al Ainy hospital effluent and Nile water samples to some different antibiotics.

Isolates			**Antibiotics**							
	sources		**Ery**	**Gen**	**Tet**	**Amp**	**Lin**	**Amo**	**Rif**	**Nal**
E. coli	**Hos. Eff.**	R	2 (3.4)	8 (16.7)	6 (10.7)	4 (7.1)	1 (1.9)	2 (3.6)	10 (17.9)	4 (7.1)
		S	54 (96.6)	48 (83.3)	50 (89.3)	52 (92.9)	55 (98.1)	54 (96.4)	46 (82.1)	52 (92.9)
	Nile water	R	0 (0)	3 (6.3)	0 (0)	0 (0)	0 (0)	0 (0)	2 (4.2)	4 (8.4)
		S	48 (100)	45 (93.7	48 (100)	48 (100)	48 (100)	48 (100)	46 (95.8)	44 (91.6)
Salmonella typhi	**Hos. Eff.**	R	3 (14.3)	2 (8.3)	4 (16.7)	3 (12.5)	3 (12.5)	1 (4.2)	8 (33.3)	1 (4.2)
		S	21 (85.7)	22 (91.7)	20 (83.3)	21 (87.5)	21 (87.5)	23 (95.8)	16 (66.7)	23 (95.8)
	Nile water	R	0 (0)	0 (0)	1 (5.6)	0 (0)	0 (0)	0 (0)	1 (5.6)	0 (0)
		S	18 (100)	18 (0)	17 (94.4)	18 (100)	18 (100)	18 (100)	17 (94.4)	18 (100)
Ps. aeruginosa	**Hos. Eff.**	R	5 (9.3)	9 (15.3)	3 (5.1)	0 (0)	8 (13.6)	4 (6.8)	13 (22)	9 (15.3)
		S	54 (90.7)	50 (84.7)	56 (94.4)	59 (100)	51 (85.4)	55 (93.2)	46 (78)	50 (84.7)
	Nile water	R	3 (6.7)	0 (0)	0 (0)	1 (2.2)	0 (0)	0 (0)	1 (2.2)	3 (6.7)
		S	42 (93.3)	45 (100)	45 (100)	44 (97.8)	45 (100)	45 (100)	44 (97.8)	42 (93.3)
Staph. aureus	**Hos. Eff.**	R	12 (33.3)	1 (2.8)	5 (13.9)	20 (55.6)	17 (47.2)	15 (41.7)	3 (0.8)	18 (50)
		S	24 (66.7)	35 (97.2)	31 (86.1)	16 (44.4)	19 (52.8)	21 (58.9)	33 (99.2)	18 (50)
	Nile water	R	39 (92.9)	0 (0)	0 (0)	12 (28.6)	6 (14.3)	12 (28.6)	1 (2.4)	13 (31)
		S	3 (7.1)	42 (100)	42 (100)	30 (71.4)	36 (85.7)	30 (71.4)	41 (97.6)	29 (69)
Strept. faecalis	**Hos. Eff.**	R	15 (46.9)	34 (100)	3 (9.4)	18 (56.2)	6 (18.8)	16 (50)	6 (18.1)	10 (31.3)
		S	17 (53.1)	0 (0)	29 (90.6)	14 (43.8)	26 (81.2)	16 (50)	26 (81.8)	22 (68.7)
	Nile water	R	4 (11.8)	0 (0)	0 (0)	16 (47.1)	2 (5.9)	10 (29.4)	4 (11.8)	12 (35.3)
		S	30 (88.2)	34 (100)	34 (100)	18 (52.9)	32 (94.1)	24 (70.6)	30 (88.2)	22 (64.7)

Notes: - ( ) = % Hos. Eff. = Hospital effluent Nal =Naldixic Acid (30 µg), Gen = Gentamycin (10 µg),
mp = Ampicillin (30 µg), Amo = Amoxicillin (30 µg), Ery = Erythromycin (15 µg),
Tet = Tetracycline (30 µg), Lin = Lincomycine (15 µg) Rif = Rifamipicin (50 µg).
R = resistant S = sensitivity

On the other hand, gram positive bacteria were more resistant than gram negative bacteria whereas the results in **Table (2)** shown, isolates belong to *Staphylococcus aureus* and *Streptococcus faecalis* isolated from Kasr AlAiny hospital effluent were resistant to Gentamycin and Ampicillin (2.8-55.6%) or (9.4-100%) for Tetracycline and Gentamycin respectively.

Staphylococcus aureus and *Streptococcus faecalis* isolated from the river Nile were very sensitive to Gentamycin and Tetracycline (100%), followed by Rifamipicin for *Staph. aureus* (97.6%) and Lincomycine for *Strept. faecalis* (94.1%).

Generally, the results in **Table (2)** showed that the bacteria isolated from Kasr AlAiny hospital effluent were more resistant to antibiotics than those isolated from the river Nile (21.1 and 9.7% respectively). This means that, different sources give different prescription resistance to antibiotics of the same microorganism against the same antibiotic tested **(Gallert *et al*, 2005)**

These results are in agreement with those obtained by **Abdullah (2005)**. Using 21 antibiotics (Tobramycin, 10 µg; Gentamycin, 10 µg; Penicillin, 10 Units; Tetramycin, 30 µg; Cefoperazone, 30 µg; Erythromycin, 15 µg; Chloramphenicol, 30 µg; Sulfa (Trimethoprim), 25 µg; Doxycyclin, 30 µg; Rifamipicin, 30 µg; Streptomycin, 10 µg; Ofloxacin, 10 µg; Cephradine, 30 µg; Ceftriaxane , 30 µg; Cefotaxime, 75 µg; Clindamycin, 30 µg; Ampicillin, 10 µg; Amoxicillin, 25 µg; Nitrofurantoin, 200 µg; Norfloxacin, 10 µg; and Carbenicillin, 100 µg).,the results in revealed that isolates of household water reservoir displayed the highest percentages of resistance (81%) to most of the antibiotics tested, followed by the isolates of mouse (72%). However, water of Ismailia Canal obtained (67%). Each of human, horse and Nile water isolates gave 62%, while swimming pool isolates, cat isolates and avian isolates showed the lowest percentage of resistance to antibiotics 57%, 47% and 34%, respectively.

Also, these results are confirmed with that obtained by **Gallert *et al*, (2005)** in Germany, more than 750 isolates of fecal Coliform (>200 strains), enterococci (>200 strains) and pseudomonades (>340 strains) from three wastewater treatment plants and from four groundwater wells in the vicinity of leaking sewers were tested for resistance against 14 antibiotics. Most, or at least some, strains of the three bacterial groups, isolated from raw or treated sewage of the three wastewater treatment plants were resistant against penicillin G, Ampicillin, vancomycin, erythromycin, triple sulfa and Trimethoprim/sulfamethoxazole (SXT).

Similarly, these results are in the line with those obtained by **Huang *et al*, (2011)** in Beijing, (China). The concentrations of penicillin-, Ampicillin-cephalothin, and Chloramphenicol-resistant bacteria were as high as $1.5x10^4$ to $1.9x10^5$, $1.2x10^4$ to $1.5x10^5$, $8.9 x10^3$ to $1.9 x10^5$ and $2.6x10^4$ to $2.0x10^5$ cfu/mL, and the average percentages in relation to total heterotrophic bacteria were 63%, 47%, 55%, and 69%, respectively. Furthermore, the authors reported that, the concentrations of tetracycline- and Rifamipicin-resistant bacteria were $6.1x10^3$ and $6.1x10^4$ cfu/mL with average percentages of 2.6% and 11%, respectively.

Our results are confirmed with that obtained in United States by **Munir *et al*, (2011)** who found that the concentrations of release of antibiotic resistant genes (tetracycline) and antibiotic resistant bacteria for tetracycline present in the final effluent of wastewater were found to be in the range of non-detectable to 2.33×10^6 copies/100 mL and $5.00x10^2$ to $10 x10^5$ cfu/100 mL respectively. In addition, the authors reported that, variations among different Wastewater Treatment Plants in the raw effluent concentration for different genes are expected because of different locations and related human activities. Also wastewater treatment plants receive inflow from a wide variety of sources beyond human population including industrial, hospital and animal waste. In addition, they reported in the literature that percentages of antibiotic resistance in a treated wastewater effluent were found to be mostly higher than the percentages in the river water and were observed to be increased downstream due to discharges from a wastewater treatment plant (**Iwane *et al*, 2001).**

Chlorine is added to wastewater and drinking water to reduce or eliminate microorganisms, which may be present in water supplies. The sensitivity of some bacteria isolated from both Kasr AlAiny hospital wastewater effluents against chlorine dose (5 ppm) in sterile Nile water throughout 120 minutes at room temperature present in **Table (3)**. Generally, the bacterial isolates from Nile water samples were more sensitive than bacterial isolates from Kasr AlAiny hospital wastewater effluent. Where the results show that, the lethal time of strains isolated from Nile water (resistant to antibiotics) during 120 minutes were 40, 30, 30, 40 and 40 minutes for *E. coli, Salmonella typhi, Pseudomonas aeruginosa, Staphylococcus aureus* and *Streptococcus faecalis*, respectively.

On the other hand, the strains isolated (sensitive to antibiotics) from Kasr AlAiny hospital wastewater effluent recorded 90, 20, 120, 120 and 120 minutes, respectively. Also, it could be noticed that *Staphylococcus aureus* and *Streptococcus faecalis* were more survival in the present chlorine (5 ppm dose for 120 minutes) than *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* especially the bacteria isolated from Kasr AlAiny hospital wastewater effluents. This means that the bacterial isolates from this hospital more resistant to chlorine than bacterial isolates from Nile water samples **(Xi *et al*, 2009).**

These results are in the line with those obtained by **Sisti *et al*, (1998**), where they exposed *Aeromonas hydrophila* to free chlorine (0.5 and 0.6 ppm) at 20 ^{o}C for 60 minutes in sterile tap water with initial counts 7.5×10^4 cfu / ml, result showed that, the counts were decreased until reaching from 10 to 70 cfu / ml. Also, **Samhan (1998)** found that, the lethal dose of chlorine (1 ppm) on some strains of *E.coli* reached after 10 minutes, while other strains showed complete inhibition after 30 minutes with contact.

Whan *et al*, (2001) Concluded that when initial inoculums level were high (10^6 cfu ml^{-1}) *Mycobacterium paratuberculosis* strain was completely killed at the free chlorine concentrations (2µg ml^{-1}) and contact times applied (30 min) in water. Also, they showed that chlorination on its own is only applied to pristine upland waters and ground waters and is usually applied in combination with other regimes. In addition, their study has highlighted the need for further research in this area in order to optimize water treatment operations and hence minimize the real and potential risk towards domestic animals and the public, respectively.

These values are comparable to those obtained by **Osman (2006)** who found the lethal time after 60 minutes when he used 4 ppm in Nile water with initial log counts 3.68 cfu / 100ml.

Table (3): Effect of chlorine dose (5 ppm) on the survival the antibiotics resistant and sensitive bacteria (cfu / 100ml) isolated from Kasr AlAiny hospital effluent and Nile water samples during 120 minutes at room temperature.

Isolates		Sources	Time (minutes) 0	10	20	30	40	60	90	120
E. coli	Resistant	Hos	3.38	2.92	2.26	1.82	1.38	078	0.3	0
		NW	3.46	2.79	1.85	1.04	0	0	0	0
	Sensitive	Hos	3.54	2.73	2.18	1.51	0.60	0	0	0
		NW	3.40	2.38	1.91	0.60	0	0	0	0
Salmonella typhi	Resistant	Hos	3.60	2.32	1.79	0	0	0	0	0
		NW	3.58	2.18	1.43	0	0	0	0	0
	Sensitive	Hos	3.46	2.08	1.34	0	0	0	0	0
		NW	3.65	2.20	1.17	0	0	0	0	0
Pseud. aeruginosa	Resistant	Hos	3.40	3.10	3.05	2.92	2.61	2.23	1.92	0.30
		NW	3.54	2.86	2.03	0	0	0	0	0
	Sensitive	Hos	3.38	3.03	2.76	2.10	1.54	0	0	0
		NW	3.66	2.91	0	0	0	0	0	0
Staph. aureus	Resistant	Hos	3.65	3.45	3.32	3.05	3.03	2.56	1.99	0.78
		Nw	3.58	3.16	2.32	1.86	0	0	0	0
	Sensitive	Hos	3.54	3.08	2.87	2.34	2.05	1.74	078	0
		NW	3.46	2.96	2.49	1.92	0	0	0	0
Strep. faecalis	Resistant	Hos	3.54	3.26	2.98	2.79	2.62	2.26	1.82	0.60
		NW	3.60	3.29	2.52	1.40	0	0	0	0
	Sensitive	Hos	3.46	3.26	2.61	2.23	1.94	1.74	0.48	0
		NW	3.54	3.22	2.62	1.68	0	0	0	0

Notes: - these number = Log number of cfu / 100ml Hos.= Hospital effluent NW = Nile water

The Rates of transfer of some antibiotics resistance features from certain antibiotics resistant bacteria isolates from Kasr AlAiny hospital wastewater effluents to antibiotics sensitive bacteria (10 isolates of each strain) from Nile water samples are shown in **Table (4).** The results demonstrated that *Staphylococcus aureus* were converted from sensitive to resistant when mixed with *Streptococcus faecalis* in sterile Nile water for 48 hours at room temperature to Erythromycin (15 µg), Gentamycin (10 µg), Ampicillin (30 µg), Amoxicillin (30 µg), and Naldixic Acid (30 µg) with ratio 20, 10, 10, 30 and 30%, respectively. Similarly, in the same pervious condition, *Streptococcus faecalis* converted from sensitive to resistant when mixed with *Staphylococcus aureus* to Gentamycin (10 µg), Ampicillin (30 µg), Amoxicillin (30 µg), Rifamipicin (50 µg) and Naldixic Acid (30 µg) with ratio 30, 10, 10, 20 and 30 %, respectively. On the other hand, *E. coli* and *Salmonella typhi* gave no complete transfer of antibiotics resistance features tested, except Naldixic Acid (30 µg) and Lincomycine (15 µg) with ratio 20 and 10 %, respectively. Also, it could be noticed that, Naldixic Acid (30 µg) resistance was transferred in all bacterial co-cultures except the mixed *Salmonella typhi* with *E. coli*.

Lebaron *et al*, (1997) found that plasmid lacking transfer functions (antibiotics resistant) from one *E. coli* K12 strain to another were investigated (sensitive) in seven sterile microcosms (biomass in aquatic environments [biofilm] soil, seawater, freshwater, wastewater, mouse gut, and mussel gut) corresponding to different environments. The plasmid transfer rates principally reflected the environmental conditions encountered in each microcosm.

Zhang *et al*, (2009 b) found that in USA 366 strain of *Acinetobacter spp*. in the wastewater were isolated from five different sites in wastewater treatment plant were determined by the disc-diffusion method for 8 antibiotics, amoxicillin/clavulanic acid (AMC), Chloramphenicol (CHL), ciprofloxacin (CIP), colistin (CL), Gentamycin (GM), rifampin (RA), sulfisoxazole (SU), and Trimethoprim (TMP).

Table (4): The availability of transfer of antibiotics resistance of some bacteria isolated from Kasr AlAiny hospital effluent to other bacteria sensitive to these antibiotics isolated from Nile water samples in sterile Nile water at room temperature for 48 hours.

Isolates: Nile water	Isolates: Hospital effluent	Ery	Gen	Tet	Amp	Lin	Amo	Rif	Nal
E. coli	*Salmonella typhi*	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	® (20)
	Pseud. aeruginosa	--- (0)	--- (0)	--- (0)	--- (0)	® (10)	--- (0)	--- (0)	® (30)
Salmonella typhi	*E. coli*	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)
	Pseud. aeruginosa	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	--- (0)	® (10)	® (10)
Pseud. aeruginosa	*E. coli*	--- (0)	® (20)	--- (0)	® (30)	--- (0)	® (20)	--- (0)	® (10)
	Salmonella typhi	® (20)	--- (0)	--- (0)	® (30)	® (20)	--- (0)	--- (0)	® (20)
Staph. aureus	*Strept. faecalis*	® (20)	® (10)	--- (0)	® (10)	--- (0)	® (30)	--- (0)	® (30)
Strep. faecalis	*Staph. aureus*	--- (0)	® (30)	--- (0)	® (10)	--- (0)	® (10)	® (20)	® (30)

Notes: - ( ) = % Nal =Naldixic Acid (30 µg), Gen = Gentamycin (10 µg) Amp = Ampicillin (30 µg)
Amo = Amoxicillin (30 µg) Ery = Erythromycin (15 µg) Tet = Tetracycline (30 µg), Lin = Lincomycine (15 µg)
Rif = Rifamipicin (50 µg). ® = resistant **---** = sensitivity

As shown in **Table (5),** the effect of chlorine dose (5 ppm) on the survival time of bacterial counts (cfu / 100ml) isolated from Nile water samples which converted from sensitive to resistant to some antibiotics during 120 minutes at room temperature. The results show that *E. coli* and *Salmonella typhi* were greatly affected by chlorination treatment. The lethal dose of chlorine was found to be 30, 30, 60, 120 and 120 minutes for *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Streptococcus faecalis*, respectively. Also, the results show that *Staphylococcus aureus* and *Streptococcus faecalis* were more survival than *E. coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* for chlorine tested before and after mixing. In addition, this study demonstrated that the behaviors of bacteria tested after transfer became more survival in chlorine dose than before.

These results are in agreement with those obtained by **Murray *et al*, (1984)** which demonstrated that the proportion of bacteria resistant to Ampicillin and cephalothin in sewage increase scantly following chlorination, and they observed a significant increase in the proportion of multidrug-resistant bacterial strains during chlorination in laboratory.

Other studies demonstrated that the susceptibility of antibiotics resistant bacteria to a disinfectant and the susceptibility of antibiotic-susceptible bacteria to a disinfectant are similar (**Rutala, 1997** & **Fraise, 2002**), indicating that disinfection does not select antibiotics resistant bacteria but instead induces the development of antibiotic resistance. On the contrary, these results are in the line with those obtained by several studies about chlorine, an agent widely used for disinfection, selects for antibiotics resistant bacteria **(Zellers, 1996, Hiraishi, 1998 and Shrivastava, 2004).**

Table (5): Effect of chlorine dose (5 ppm) on the survival of the converted antibiotics resistant bacteria (cfu / 100ml) isolated from Nile water samples during 120 minutes at room temperature.

Time (min.)	Log number of cfu / 100ml: *E. coli*	*Salmonella typhi*	*Pseud. aeruginosa*	*Staph. aureus*	*Strept. faecalis*
0	3.60	3.66	3.46	3.58	3.54
10	2.41	2.22	2.69	3.24	3.02
20	1.34	1.04	2.45	2.66	2.54
30	0.65	0.33	2.01	2.24	2.02
40	0	0	1.22	1.88	1.68
60	0	0	0.78	1.65	1.22
90	0	0	0	1.52	1.02
120	0	0	0	1.02	0.90
Mean	1.00	0.91	1.58	2.22	1.99

So, this study introduces some information about the role of bacteria which reached to the Nile water from wastewater (especialy of the hospital effluent) in converting bacteria in Nile water from sensitive to some antibiotics to another set of bacterial strains resistant to some antibiotics and survival for 120 minutes in 5 ppm of chlorine dose. In addition, this study shed why some light on the reasons some bacteria more resistant to disinfection by chlorine in Wastewater and Drinking Water Treatment Plants as well as the water quality produced.

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